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Use of the Photo-Electromyogram to Objectively Diagnose and Monitor Treatment of Post-TBI
Light Sensitivity

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14. ABSTRACT <u>Purpose:</u> to test the whether photosensitivity (photophobia) after traumatic brain injury (TBI) is due to increased sensitivity of the brainstem trigeminal sensory nucleus, as revealed objectively by an exaggerated photoblink reflex (photo-electromyogram). This will be tested in humans and in a mouse strain genetically engineered to be hypersensitive to calcitonin gene related peptide (CGRP), the neurotransmitter modulating trigeminal nerve function. <u>Scope:</u> objective methods to quantify photo-sensitivity include 1) light evoked potentials (electromyogram) from the blinking and squinting muscles of the forehead 2) the pupil light reflex 3) light evoked changes in sympathetic nerve activity, measured by changes in skin conductance and heart rate. <u>Major Findings (Year 2):</u> 1) First successful recording from EMG electrodes implanted into the mouse orbicularis oculi muscle with wireless transmission of EMG in response to stimuli in an awake, unanesthetized mouse, 2) Observation of increased orbicularis EMG activity in response to increased light levels, 3) Observation of an apparent enhancement of an air puff induced blink by light after administration of an intraperitoneal injection of calcitonin gene related peptide (CGRP). <u>Significance:</u> objective testing of photosensitivity in humans and mice will provide new approaches to finding the underlying mechanisms, classification of photosensitivity, diagnosis and monitoring of new treatments.					
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INTRODUCTION

Two of the most prevalent problems reported by military personnel following traumatic brain injury (TBI) are photosensitivity and headache. Currently, better means are needed for diagnosing and treating post-traumatic light sensitivity and headache. The goal of this project is to establish a clinically translatable assay of photosensitivity to facilitate diagnosis and treatment of light sensitivity and headache. The clinically translatable assay will take advantage of a natural brain reflex, the photic-electromyogram (EMG), a reflex contraction of the eyelid muscles in response to light. The photic EMG is modulated by the thalamus and central sensory trigeminal pain center of the brainstem, which conveys light input to the facial muscles to elicit an eye blink and squinting response. We hypothesize that the hallmark of patients with photosensitivity is abnormal sensitization of the thalamus and brainstem trigeminal neurons to light input. This grant's objective is to show that the trigeminal and photic blink reflex to light, as measured by the photic EMG, is a valid surrogate for assessing central thalamic and trigeminal hypersensitivity as a cause for photosensitivity and headache, which can be treated. The specific aims of this grant are twofold: 1) to objectively characterize the photosensitive response in humans by recording the photic EMG in normal subjects compared to photosensitive patients and assess treatment with blue blocking lenses, and 2) to examine the photosensitive response in awake, un-anesthetized mice by recording the photic EMG in a genetic mouse strain that has trigeminal hypersensitivity and light aversion. The effect of injecting calcitonin gene related peptide (CGRP), the neurotransmitter modulating trigeminal neurons, and an antagonist, olcegepant, will be used to investigate a new medical treatment of photosensitivity in the mouse model. These studies will establish the foundation for future clinical diagnosis and treatment of photosensitivity.

BODY – RESEARCH ACCOMPLISHMENTS ASSOCIATED WITH APPROVED STATEMENT OF WORK FOR YEAR 2:

1) IRB and Animal Use submission

In Year 2, we were notified of approval of our human use protocol from the DOD Human Use Officer in the following memo (animal protocol approval occurred at the end of Year 1):

Classification: UNCLASSIFIED

Caveats: NONE

SUBJECT: Initial Approval for the Protocol, "Use of the PhotoElectromyogram to Objectively Diagnose and Monitor Treatment of Post-TBI Light Sensitivity," Submitted by Randy H. Kardon, MD, PhD, University of Iowa and Veterans Affairs Health Care System, Iowa City, Iowa, Proposal Log Number 11125001, Award Log Number W81XWH-11-1-0561, HRPO Log Number A-17005

The HRPO point of contact for this study is Lori J. Walther, Human Subjects Protection Scientist, at 301-619-2286/lori.j.walther.ctr@us.army.mil.

CARYN L. DUCHESNEAU, BS, CIP
Chief, Human Subjects Protection Review
Human Research Protection Office
Office of Research Protections
US Army Medical Research and Materiel Command

After receiving approval for the human use portion of the study, we have begun to identify patients with photosensitivity. We are accessing a TBI database from the Iowa City VA Medical Center as a source for military- associated TBI and also patients are being identified that have photosensitivity after TBI which are referred to the Iowa City VA Eye Clinic are being seen by the PI (Randy Kardon MD PhD) in his VA and University of Iowa neuro-ophthalmology clinics. These patients are being recruited for the study. In addition, we are accessing the University of Iowa Hospital patient database by diagnosis allowing us to obtain an Excel spreadsheet listing patients with TBI and also those with photosensitivity as a diagnosis. Patients identified from the VA and UIHC database are being sent letters of introduction about the study and are contacted by phone. We also have a list of normal subjects that we have used as research subjects for other studies and will be recruiting normal subjects from this pool, since their visual system has already been well characterized. Finally, we have also been recruiting migraine patients since they commonly report light sensitivity between headaches and are recruiting migraine subjects in the immediate 25-mile radius as subjects using email announcements and also the UIHC database by diagnostic category and patient location.

We have already successfully obtained approval for the Animal Use component of the study from our local animal use committee and also from the DOD.

We have been in communication with Brad C Motter PhD and Mary M. Jackowski OD, Syracuse VA and SUNY Upstate Medical Center University, who have been conducting pilot research on treatment of photosensitive patients using two independent treatments 1) constriction of the pupil using 1% pilocarpine and 2) use of a fixed pupil aperture contact lens which blocks out any light outside of the artificial pupil (using a 4.5 mm diameter artificial pupil). In their preliminary work, using a validated questionnaire to quantify subjective light sensitivity, they found a significant therapeutic effect with both treatments. What was unusual was that the group of

subjects treated in each eye with one drop of pilocarpine 1% to constrict their pupils, had a long-lived effect for weeks, much longer than the miotic action of the topical pilocarpine (which usually lasts for only hours). Because of these results, Drs. Motter and Jackowski have agreed to collaborate with us to further test these treatments using their questionnaire, but also using our method of quantifying orbicularis and procerus EMG responses to light. We intend to submit a modification of our human protocol this next quarter so that we can also try these treatments in our controlled experimental setting.

We already have had an approved IRB3 for a pilot study that has tested human EMG responses from the eyelid and forehead muscles as a function of light intensity. This had been performed in a limited number of normal subjects, migrainers and patients with traumatic brain injury who report light sensitivity. We are taking advantage of the pilot project experience to help refine the testing protocol and analysis of the EMG signals recorded that we will also be using as an outcome measure for the present DOD funded project.

We have also recently been able to acquire space in the ophthalmology clinic to conduct the human testing that is within close proximity to the patient area so that the human testing portion can be performed without patients having to travel outside of the ophthalmology clinic for the testing.

2) Optimization of a novel method to objectively assess photosensitivity in humans and mice (months 1-24)

Statement of Work (SOW)

Task 1a. Optimization of hardware and software interface for human recording of wireless electromyogram (EMG) elicited by light from the eyebrow and eyelid muscles using “dry” low impedance surface skin electrodes (Sigmed, Inc.).

In the last quarter of 2012, we successfully optimized the hardware (light stimulation with large dynamic range LED/LCD monitor, EMG, heart rate recording, pupil responses, and skin conductance recording) and have written software that is able to control each component of hardware and these are all time stamped so that each piece of recording can be related to one another (and to the light stimulus) over time. In the first quarter of 2013, the PI has developed plans for a molding that will conform to each subject's face, and that will allow us to mount the soft, dry wireless EMG electrodes on it so that they will make gentle contact with the skin overlying the blinking and squinting muscles. This will replace the standard skin electrodes that are adhesive and require skin preparation.

Over quarters 2-4, we have iteratively formalized and started implementation of the fabrication of a headset that will hold the dry wireless electrodes on the skin overlying the orbicularis and procerus muscles for recording EMG responses to light. This has been in conjunction with Mark Ginsberg, owner of Ginsberg Jewelry in Iowa City and Bounnak Thammavong, his design assistant, to develop a molding which can be adjusted to change the tension between the electrodes and the skin and which can be rendered using a 3D printer. This will replace the standard skin electrodes that are adhesive and require skin preparation. This local group has the expertise that we need for developing a 3D CAD model and printing of the molding to which we will mount the electrodes. The 3D printer can print the molding on-site using a flexible and soft polymer resin, whose properties we can specify, including a polymer that can be autoclaved for sterilization. An example of the CAD design of a prototype is shown below in different views (Figure 1):

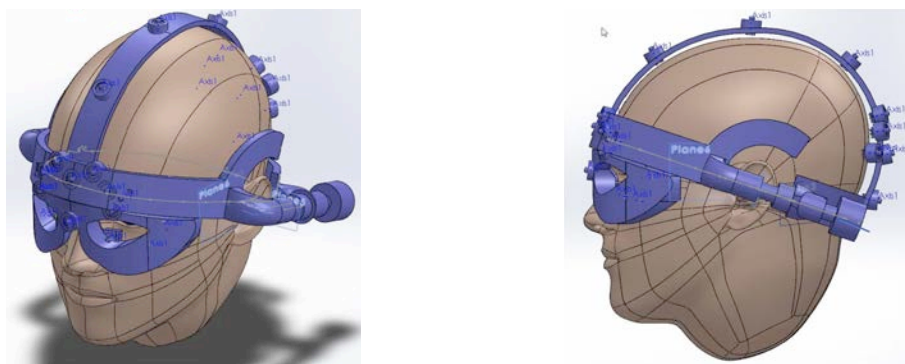


Figure 1: 3D CAD rendering of head/face mount for EMG wireless electrodes that is in the process of being finalized. The end product will be more streamlined, lightweight, and will not be as bulky as shown here. Once finalized, the printing of the headset will be fast and can be repeated easily for multiple configurations to accommodate different sized and shaped heads.

Task 1b. Integration of software with EMG recording to simultaneously record and analyze skin conductance, beat-to-beat variation in the electrocardiogram (ECG), and pupil

We have successfully developed the software for analysis of the recorded EMG responses, which is the main outcome measure. Previously we were measuring the maximal response to light, but we have implemented the software to quantify the changes in the beat to beat variations in heart rate in response to light stimuli of varying intensity, duration and color. In addition, we have implemented software to calculate the area under the rectified EMG signal obtained from both human and mouse measurements. This is a critical metric that we can now measure to reflect any sustained EMG response, independent of the maximal RMS amplitude of EMG response to light. In our pilot data in mice and humans we found that the sustained response was very susceptible to the disease state being measured (light sensitivity), so this measurement will be an important outcome measure, which we can now quantify.

Task 1c. EMG recording from chronically implanted electrodes in the mouse will be developed using the DSI wireless system of data transmission of biopotentials.

The first attempt at chronically instrumenting mice was accomplished in the first quarter of Year 2, using the DSI wireless data transmission system. We have implanted a functional transmitter into a subcutaneous pocket beyond the scapulae on the dorsal flank of a transgenic nestin/hRAMP1 mouse that shows photosensitivity. The lead wires were inserted into the superior fibers of the orbicularis oculi and the animal was allowed to recover from the surgery. Following recovery, we were successfully able to measure EMG responses at rest in the light and dark, and in response to stimuli, such as an air puff (Figure 2) in an awake, free roaming mouse. Our primary focus has been to determine if we can record an EMG response to light in conscious mice. Initial results are very encouraging (Figure 3). Further refinements are being made to reduce noise from EMG signals generated by mouse movement. To reduce this problem we have constructed a small transparent Plexiglas chamber that minimizes lateral and vertical movement of the mouse without restraint. In addition to spontaneous EMG activity, we have used an air puff to induce a blink response and to measure the interaction of light with this reflex response. While still preliminary, the induced blink response appears to be more prolonged in the mouse when combined with bright light compared to the dark (Figure 4). This was also confirmed by visual observation of the mouse. Initial studies with an intraperitoneal

injection of calcitonin gene related peptide (CGRP), the neurotransmitter of the trigeminal nerve, suggest that the response after CGRP may be even greater in the light, which is encouraging. However, caution is needed since quantitation of the data and further studies with additional mice will be necessary to substantiate these initial positive findings.

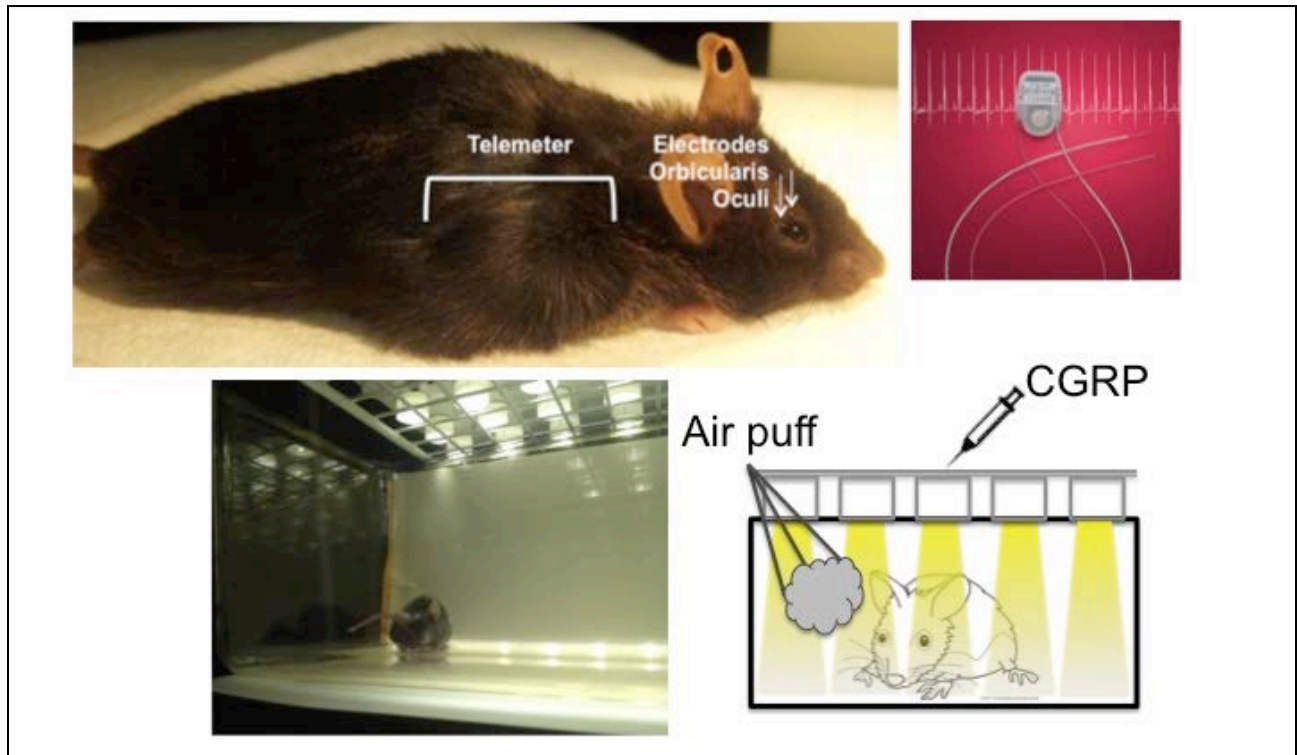


Figure 2. Experimental paradigm for measuring photic-induced EMG responses in the orbicularis muscles of the mouse. The positions of the wire-less telemeter and recording electrodes (unit shown at top left) are indicated in the mouse shown in the top image. The mouse is shown in the recording cage (bottom images) with a schematic indicating stimulation by an air puff, bright light, or CGRP injection.

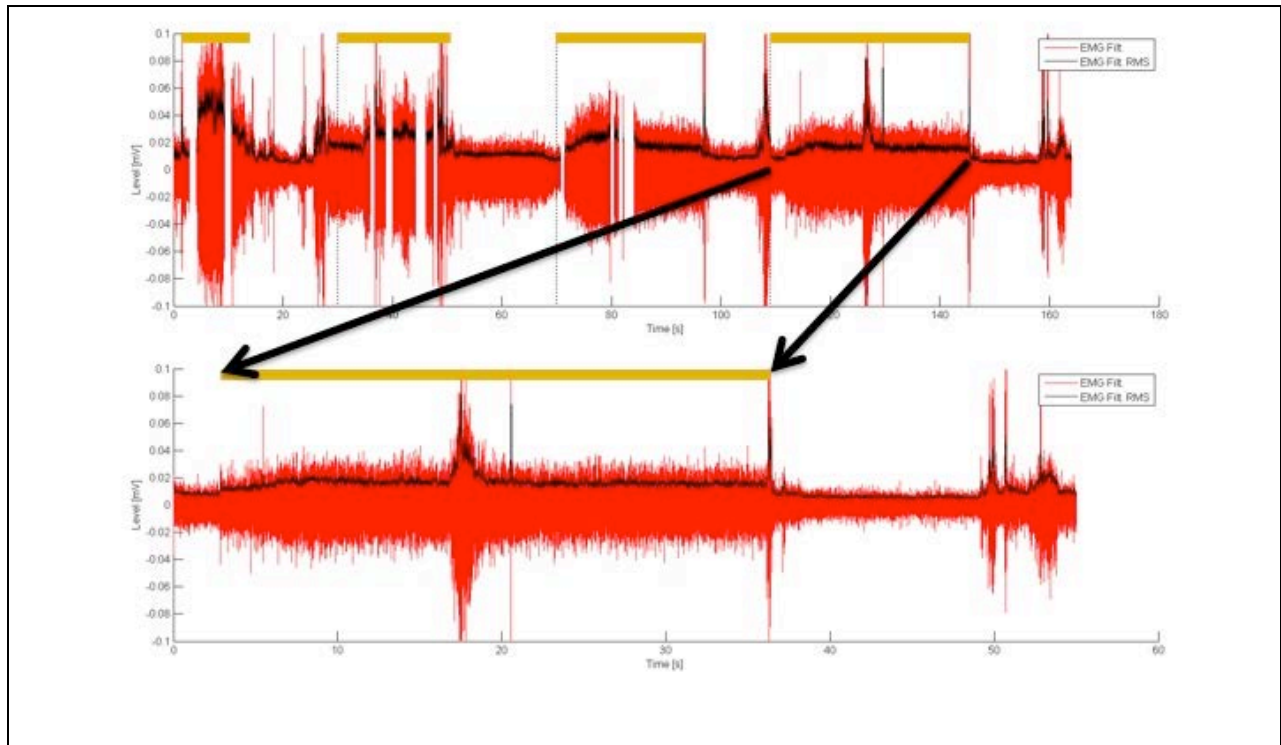


Figure 3. Increase in orbicularis EMG activity in response to light. A representative bipotential EMG tracing from a nestin/hRAMP1 mouse is shown, with the mouse either in the dark or bright light (27,000 lux). The light periods are indicated by the yellow bars. Note how the EMG response increases during the light periods. A magnified view is shown in the bottom image.

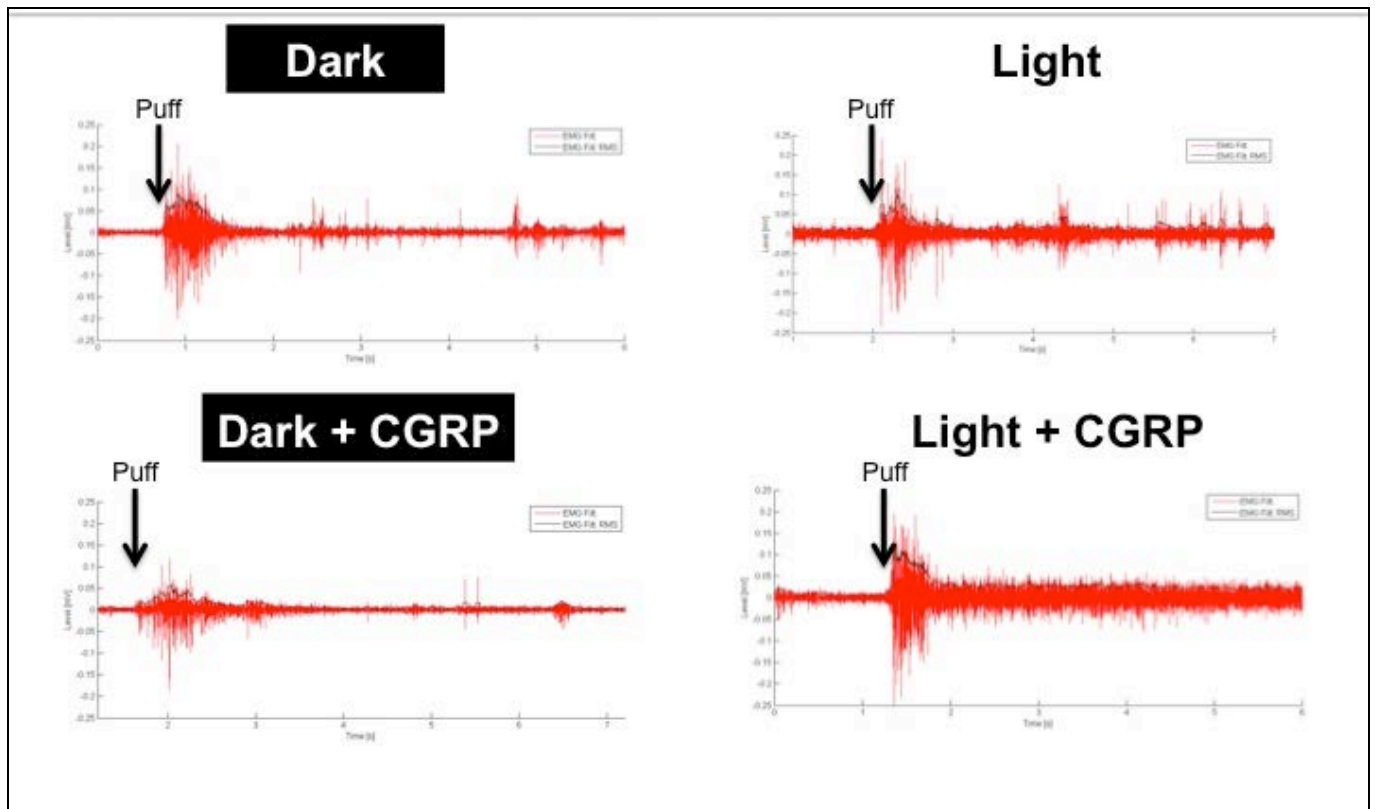


Figure 4. Apparent enhancement of an air puff induced blink by light and CGRP administration. The same mouse was exposed to a gentle air puff (indicated by arrow) either in the dark or bright light (27,000 lux) (top panels). The bottom panels are following an ip injection of CGRP. Representative tracings of the bipotential EMG response are shown.

During the latter part of Year 2, we have also successfully engineered a system to precisely control the duration and timing of the air puff corneal stimulus using a sensitive microphone. Additionally, we have engineered a light sensor so that any light stimulus applied can be precisely recorded with a time stamp. This will allow us to accurately measure the latency, amplitude and duration of the EMG responses in the awake freely moving mouse with respect to stimuli applied. Another important advantage of having a time stamp for the different stimuli is that in the case of repeated stimuli (i.e. in mouse experiments), we will be able to align and average response waveforms together (Figure 5).

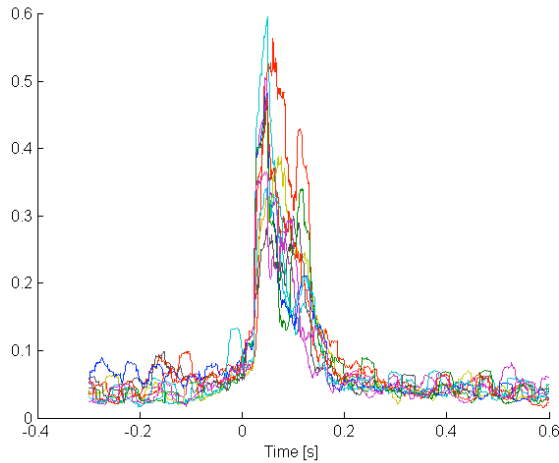


Figure 5. Example of multiple air puffs directed at a conscious mouse cornea in an animal chronically instrumented with EMG electrodes. Each color is a separate air puff run. Using the time stamp, all of the different responses to each experimental run can now be superimposed and averaged to provide a more robust signal/noise ratio. This will allow a better quantification of response, even to milder stimuli. X axis is time and Y axis is EMG amplitude in millivolts.

The system to deliver an air puff stimulus to our mice for the EMG experiment is activated by the push of a red button (shown in Figure 6 below). This causes air to be released from a solenoid-activated valve, which allows an air puff to be delivered through polyethylene tubing, which splits to form a Y intersection. With equal tubing lengths for both arms of the Y, the two resulting air puffs reach the end of the tubes simultaneously. One air puff is delivered to the conscious mouse in a clear Plexiglas housing and elicits a reflex blink while the other air puff in the second branch of the Y tubing is delivered to a microphone simultaneously to produce a time stamp of when the puff is delivered.

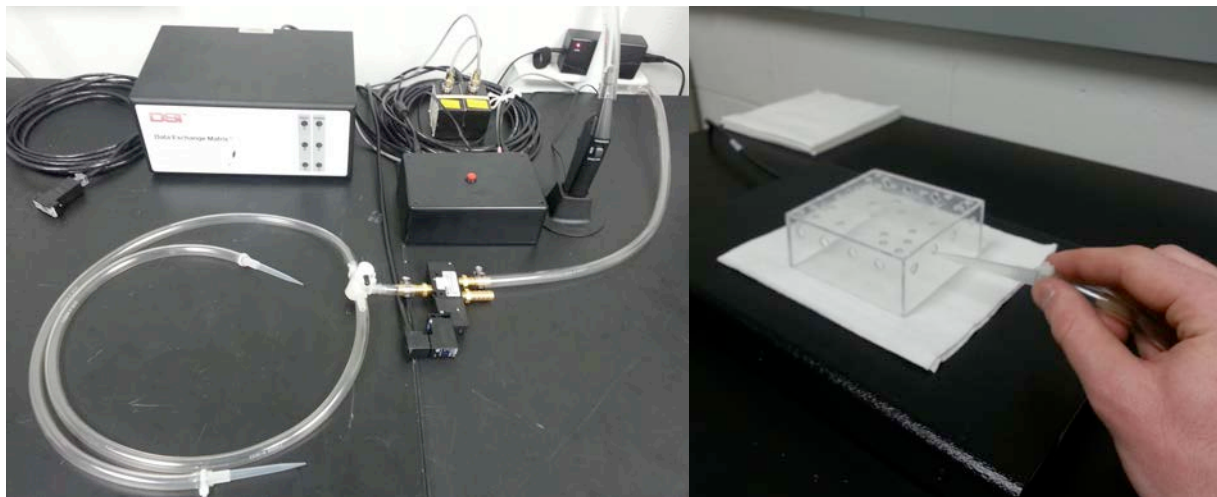


Figure 6. System for delivering air puffs to the eye of awake mice and simultaneous delivery to a microphone to record the timing of the air puff. In the left of the figure is the Plexiglas housing for the mouse, which can be used to deliver air puffs for the corneal blink reflex and light stimuli.

In conjuncture with the audio time stamp, the system also records when the circuit is completed

as the button is pressed. Both can be observed simultaneously along with the EMG recording, mouse temperature, and signal strength.

During the 4th quarter of Year 2, we have spent a major effort to refine the surgical implantation technique for chronic recording of the EMG from eyelid muscles in the mice. The following summarizes our progress:

Anesthesia (currently): A switch was made from ketamine/xylazine to isoflurane for anesthesia during the electrode implantation for chronic recording of EMG. A concentration of 5.0% isoflurane and 1.5% oxygen is used to induce the mouse while a maintenance concentration of 3.5% isoflurane and 1.5% oxygen is used during surgery. Isoflurane is much more effective at fully anesthetizing the mice as well as providing a speedy recovery time (~10 minutes). The method is also much more effective at keeping mice anesthetized for longer surgeries such as this. With ketamine/xylazine, mice larger than ~25 g were difficult to fully anesthetize and thus were omitted from use. Using isoflurane, larger mice can and have been effectively used for implantation. Their larger size helps accommodate the implant more so than smaller mice.

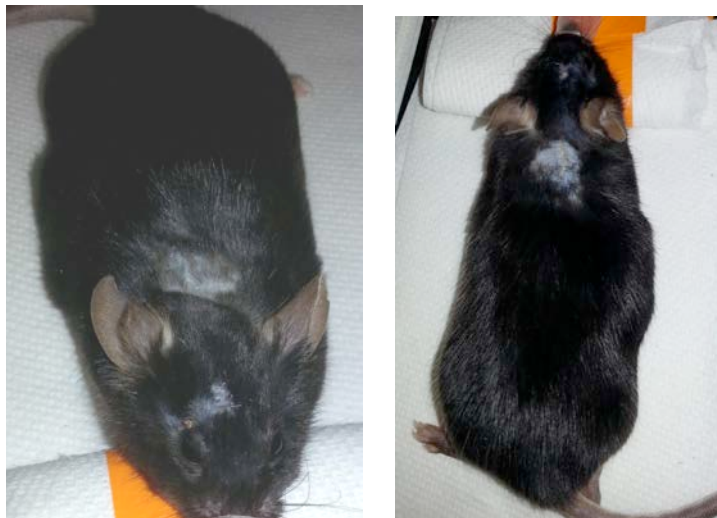


Figure 7. Recovery of mouse chronically implanted with subcutaneous EMG electrodes and transmitter. There was no re-opening of incisions or excoriation of adhesive. Also, there were no signs of necrosis around the transmitter. Electrodes can be observed just over the eye. The larger sized mouse appears to be more accommodating for the transmitter and less invasive.

Incisions and suturing:

Surgical Procedure (currently): Instead of making one large incision over the top of the skull, two smaller incisions are made: one small midline incision just superior to the eyes and one midline incision between the scapulae. The skin over the top of skull remains uncut. The caudal incision is just large enough for the transmitter to pass through while the rostral incision is just large enough for the insertion of the electrodes into the orbicularis oculi muscles. A trocar and sleeve is used to tunnel between incisions and thread the electrode wires. The two incisions are sutured using dissolvable 7-0 coated Vicryl suture to prevent further irritation. Not completely incising the area over the skull eliminated much of the surgical trauma and has facilitated rapid healing and recovery from the surgery. The tunnel made by the trocar keeps the wires in place more effectively as well as providing a smaller area for the adhesive to cover, allowing the possibility for intra-ventricular injections of CGRP or its antagonists, if we desire this

in the future. The wires are also showing no signs of being excised from the mouse thus far, providing additional time for chronic testing over time (Figure 7).

Task 2 (Year 2). In normal humans without photosensitivity, collect and define the normative range of values for light induced EMG, pupillary light reflex, skin conductance, and ECG in response (months 13-24):

2a. Collect data on 50 normal subjects with optimized protocol for testing light induced outcomes as in Task 1; 25 subjects without history of TBI and 25 post TBI normals (months 13-24)

2b. Analyze pupil light reflexes, skin conductance, ECG, and EMG (months 13-24)

Because of difficulties in identifying adequate space and location to accommodate our human subjects, the human testing was delayed in Year 2. Since we recently were able to reconfigure human testing space within the ophthalmology clinic in this last quarter (Figure 8), we can now start recruiting subjects to begin testing. We have identified normal human subjects and those with TBI from our database patient and subject files and we are now in the process of scheduling their testing.



Figure 8. New clinical testing space recently acquired within the confines of the Ophthalmology Clinic. The test subject sits comfortably in front of a large screen which can present a large range of light stimuli, varying in color, intensity and size which are used to elicit objective responses such as EMG of the orbicularis and procerus muscles, skin conductance and heart rate.

Task 4 (Year 2). In normal littermate mice and in mice rendered photosensitive (genetically altered to over-express the receptor for (CGRP), compare the EMG response to light with intra-ventricular vehicle injection vs CGRP (months 13-24):

4a. Record and analyze eyelid EMG responses to light in normal littermate control mice of the hRAMP1 strain, comparing vehicle with intra-ventricular CGRP (months 13-24).

4b. Record and analyze eyelid EMG responses to light in genetically altered hRAMP1 photosensitive mice, comparing vehicle with intra-ventricular CGRP (months 13-24).

As stated above, in the last quarter we have successfully computerized the air puff delivery system and using a microphone we can now time stamp every air puff delivery. Similarly, with a light sensor incorporated into the mouse test environment, we can now time stamp any light stimulus given by itself or in conjunction with an air puff stimulus to the cornea. We have instrumented 3 more mice with EMG electrodes. However, over time the mice have pulled out the wires that were anchored to the skull under the skin. We have therefore modified our surgical approach during implantation to try and prevent this from occurring (see above).

KEY RESEARCH ACCOMPLISHMENTS (SUMMARY)

- Implementation of software to calculate the area under the rectified EMG signal obtained from both human and mouse measurements to reflect any sustained EMG response, independent of the maximal RMS amplitude of EMG response to light.
- Assembly, integration and testing of a system to precisely control the duration and timing of the air puff corneal stimulus using a sensitive microphone, and a light sensor so that any light stimulus applied can be precisely recorded with a time stamp.
- First successful recording from EMG electrodes implanted into the mouse orbicularis oculi muscle with wireless transmission of EMG in response to stimuli in an awake, unanesthetized mouse.
- Observation of increased orbicularis EMG activity in response to increased light levels.
- Observation of an apparent enhancement of an air puff induced blink by light after administration of an intraperitoneal injection of calcitonin gene related peptide (CGRP).
- Successful modification and testing of the surgical protocol for implanting the wireless EMG electrodes into the mouse orbicularis oculi muscle, including 1) a switch from ketamine/xylazine to isoflurane for anesthesia during the electrode implantation, and 2) replacing one large incision over the top of the skull with two smaller incisions: one small midline incision just superior to the eyes and one midline incision between the scapulae, leaving the skin over the top of skull uncut, and thus minimizing re-opening of incisions, excoriation of adhesive and necrosis from occurring.

REPORTABLE OUTCOMES

Presentation of pilot data as a poster on the Photo-EMG in humans (Carver University of Iowa internal pilot grant) at the Association of Research and Vision in Ophthalmology (ARVO), Ft. Lauderdale, FL May 2013.

Presentation of preliminary results in an invited talk for Veterans Vision and TBI Symposium at the Association for Research in Vision and Ophthalmology International Meeting, Seattle, WA May 2013.

Presentation of preliminary results as invited Speaker and Instructor at VA and DOD sponsored course and meeting on Visual Dysfunctions Associated with Traumatic Brain Injury (TBI) (in conjunction with the Blinded Veterans Association), August 2013, Spokane, Washington.

Presentation of preliminary results as Visiting Professor, Stony Brook University, Department of Ophthalmology May 2013.

Presentation of results as platform paper given to the North American Neuro-ophthalmology Society Annual Meeting, Feb 2013.

CONCLUSIONS

The research work that we are carrying out has important implications for the greater public good, in addition to its military relevance. Light sensitivity and migraine headaches following traumatic brain injury are the two most commonly reported symptoms in military personnel exposed to direct trauma to the brain or indirectly from blast injury. Similar symptoms can also occur in the civilian population from TBI resulting from motor vehicle accidents and also from head injury due to contact sports at both the school and professional level. At present there are no biological markers or tests that can be used to objectively diagnose and monitor treatment of photo-- sensitivity or migraine headaches. This would be the first research to facilitate investigations of the mechanisms in humans using controlled, photic stimuli with monitoring of physiological reflexes in response to the light stimuli. In order to accomplish this task, it is required that a sophisticated software and hardware integration be in place to accurately measure light evoked reflexes that can be used in research and in a clinical setting. In addition, adding the capability of studying the photic EMG in conscious mice will provide an important scientific platform upon which to use genetic and drug investigations on the mechanism of light sensitivity and migraine and new treatments.

REFERENCES – a literature search was performed to update the previous literature on photophobia associated with TBI and yielded the following relevant references:

- 1: Bulson R, Jun W, Hayes J. Visual symptomatology and referral patterns for Operation Iraqi Freedom and Operation Enduring Freedom veterans with traumatic brain injury. *J Rehabil Res Dev.* 2012;49(7):1075-82. PubMed PMID: 23341280.
- 2: Alvarez TL, Kim EH, Vicci VR, Dhar SK, Biswal BB, Barrett AM. Concurrent vision dysfunctions in convergence insufficiency with traumatic brain injury. *Optom Vis Sci.* 2012 Dec;89(12):1740-51. doi: 10.1097/OPX.0b013e3182772dce. PubMed PMID: 23190716.
- 3: Vadapalli SP. Color-enhanced tinted contact lenses in the visual rehabilitation of a mild traumatic brain injury patient. *Insight.* 2012 Summer;37(3):20-1. PubMed PMID: 22970482.
- 4: Capó-Aponte JE, Urosevich TG, Temme LA, Tarbett AK, Sanghera NK. Visual dysfunctions and symptoms during the subacute stage of blast-induced mild traumatic brain injury. *Mil Med.* 2012 Jul;177(7):804-13. PubMed PMID: 22808887.
- 5: Belliveau MJ, Jordan DR. Relief of refractory photo-oculodynia with botulinum toxin. *J Neuroophthalmol.* 2012 Sep;32(3):293. doi: 10.1097/WNO.0b013e3182585b5d. PubMed PMID: 22549562.
- 6: Digre KB, Brennan KC. Shedding light on photophobia. *J Neuroophthalmol.* 2012 Mar;32(1):68-81. doi: 10.1097/WNO.0b013e3182474548. Review. PubMed PMID: 22330853; PubMed Central PMCID: PMC3485070.

APPENDICES – none

SUPPORTING DATA – all figures including in body of report